Intensity of acute exercise does not affect serum leptin concentrations in young men

By: A. Weltman, C. J. Pritzlaff, L. Wideman, R. V. Considine, D. A. Fryburg, M. E. Gutgesell, M. L. Hartman, and J. D. Veldhuis

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Abstract:

Purpose: We examined the effects of exercise intensity on serum leptin levels.
Methods: Seven men (age = 27.0 yr; height = 178.3 cm; weight = 82.2 kg) were tested on a control (C) day and on 5 exercise days (EX). Subjects exercised (30 min) at the following intensities: 25% and 75% of the difference between the lactate threshold (LT) and rest (0.25 LT, 0.75 LT), at LT, and at 25% and 75% of the difference between LT and $\dot{V}O_2 peak$ (1.25 LT, 1.75 LT).
Results: Kcal expended during the exercise bouts ranged from 150 ± 11 kcal (0.25 LT) to 529 ± 45 kcal (1.75 LT), whereas exercise + 3.5 h recovery kcal ranged from 310 ± 14 kcal (0.25 LT) to 722 ± 51 kcal (1.75 LT). Leptin area under the curve (AUC) (Q 10-min samples) for all six conditions (C + 5 Ex) was calculated for baseline (0700–0900 h) and for exercise + recovery (0900–1300 h). Leptin AUC for baseline ranged from 243 ± 33 to 291 ± 56 ng~mL^{-1} X min; for exercise + recovery results ranged from 424 ± 56 to 542 ± 99 ng~mL^{-1} X min. No differences were observed among conditions within either the baseline or exercise + recovery time frames. Regression analysis confirmed positive relationships between serum leptin concentrations and percentage body fat ($r = 0.94$) and fat mass ($r = 0.93$, $P < 0.01$).
Conclusion: We conclude that 30 min of acute exercise, at varying intensity of exercise and caloric expenditure, does not affect serum leptin concentrations during exercise or for the first 3.5 hours of recovery in healthy young men.
Key Words: LEPTIN, EXERCISE, GROWTH HORMONE, LACTATE THRESHOLD

Article:

Leptin, the product of the adipocyte *ob* gene is a secreted hormone that communicates to the brain the amount of adipose tissue in the body (5). The greater the amount of adipose tissue present, the more leptin is produced and released into the circulation (5). Although serum leptin levels are most strongly related to the amount of adipose tissue in the body (4), several other factors have also been shown to influence serum leptin concentrations (4,21). For example, weight loss results in a reduction (5,19,21,28) and weight gain in an increase in leptin levels (16). Serum leptin falls dramatically with short-term fasting (in the absence of weight loss) (15,28) and is elevated as a result of 1 d of massive overfeeding (16). Leptin production is also affected by gender, metabolic hormones, and pharmacological agents (4,21).

Exercise is a potent stimulus for secretion of many hormones (7,8,25,30) and has been suggested to influence serum leptin levels (11,13,17,22,24). Although exercise training has reduced serum leptin levels (11, 13,22,24), the effects of exercise, independently of loss of fat mass, have not been clearly established. In the limited number of studies where acute exercise was examined (6,10,17,24,26), short-term exercise had no effect on serum leptin levels. In contrast, recent data suggest that prolonged exercise (marathon run, ultramarathon, 3-h cycling) may result in a fall in serum leptin levels (14,17,18). This reduction in serum leptin with exercise may be related in part to the time of exercise and whether subjects are fed or fasted (14). These data suggest that a "critical" level of exercise mediated energy expenditure may be needed in order for serum leptin levels to be lowered by acute exercise.

However, in the above studies, the effects of exercise intensity on serum leptin release were not examined. Exercise intensity is strongly related to the release of other metabolic hormones, including catecholamines (20,30) and growth hormone (GH) (7,25), with increasing intensity of exercise resulting in an increase in the magnitude of hormonal release. Because it has been suggested that leptin is a metabolic signal that regulates GH secretion (3), and based on our recent findings of a linear dose response relationship between exercise intensity and GH release (25), we hypothesized that exercise intensity may also have an impact on serum leptin levels. In the present study, we compared the effects of various exercise intensities on leptin release during 30-min of exercise and 3.5 h of recovery thereafter.

METHODS

Subjects. Seven recreationally active men (mean age (± SEM) = 27.0 ± 1.1 yr; mean height, 178.3 ± 6.5 cm; mean weight, 82.2 ± 2.9 kg) provided voluntary written informed consent, as approved by the Human Investigation Committee of the University of Virginia, before entering the study. Each subject underwent a detailed medical history and physical examination, and no subject had a history of pituitary, renal, hepatic, or metabolic disease. Screening laboratory data revealed normal hematologic, metabolic, renal, hepatic, gonadal, and thyroid function. Subjects were nonsmokers and non-heavy alcohol users and refrained from exercise for 24 h before each evaluation.

Experimental design. Each volunteer completed a treadmill test to assess level of cardiovascular fitness and underwater weighing for determination of body density at the Exercise Physiology Laboratory of the University of Virginia General Clinical Research Center (GCRC). Subjects were then evaluated on six separate occasions, five with exercise and one at rest. The order of the six study conditions was assigned in a randomized fashion. The admissions were scheduled at least seven days apart and no more than two admissions per 2 months were allowed. Exercise consisted of 30-min of constant load exercise at a predetermined velocity. Velocities (V) were set as the V associated with 25% and 75% of the difference between the oxygen consumption ($\dot{V}O_2$) at the lactate threshold (LT) and ($\dot{V}O_2$) at rest (.25LT and .75LT, respectively); the V at LT (LT); and the V at 25% and 75% of the difference between the ($\dot{V}O_2$) at LT and $\dot{V}O_2$peak (1.25LT and 1.75LT, respectively). The use of 30-min exercise bouts was chosen to simulate typical and recommended exercise duration (1).

Body composition. Body density was assessed by hydrostatic weighing (12). Each subject was weighed in air on an Accu-weigh beam scale (Metro Equipment, Sunnyvale, CA) accurate to 0.1

kg and subsequently weighed underwater on a Chatillon (New York, NY) autopsy scale accurate to 10 g. Residual lung volume was measured using an oxygen- dilution technique (31). The computational procedure of Brozek et al. (2) was used to determine relative fat from body density measurements.

$\dot{V}O_2$peak. A continuous treadmill (Quinton Q 65 treadmill, Seattle, WA) exercise protocol was used to measure LT and ***$\dot{V}O_2$peak***. The initial velocity was set at 100 m•min^{-1} with increases in velocity of 10 m•min^{-1} every 3 min. Open circuit spirometry was used to collect metabolic data (SensorMedics Model 2900Z metabolic measurement cart, Yorba Linda, CA). Heart rate was determined via a Marquette Max-1 electrocardiograph (Marquette, WI). An in-dwelling venous cannula was inserted in a forearm vein 0.5 h before the test, and blood samples were withdrawn at rest and during the last 15 s of each stage for the measurement of blood lactate concentration (Yellow Springs Instruments (YSI) 2700 Select Biochemistry Analyzer, Yellow Springs, OH). Test termination occurred when the subjects reached volitional exhaustion. ***$\dot{V}O_2$peak*** was chosen as the highest O_2 consumption ($\dot{V}O_2$) attained.

Determination of the lactate threshold. The blood lactate–velocity relationship obtained from the ***$\dot{V}O_2$peak*** /LT test was used to determine the LT. Velocity at LT was determined by plotting blood lactate concentration against treadmill velocity and was chosen as the highest velocity obtained prior to the curvilinear increase in blood lactate concentration with increasing velocities. An elevation in blood lactate concentration of at least 0.2 mM (the error associated with the lactate analyzer) above baseline was required for LT determination. $\dot{V}O_2$ associated with velocity LT was then determined (29).

Exercise/control days. Subjects were admitted to the GCRC on the evening before the exercise/control studies. Subjects were required to consume their evening meal at or before 1700 h and then received a standardized snack at 2000 h. The nutrient composition of the snack was 55% carbohydrate, 15% protein, and 30% fat. Subjects were allowed to consume water ad libitum. To avoid the confounding effects of meals on leptin release, subjects then fasted until the end of the admission. At 2100 h, intravenous cannulas were placed in veins of both forearms. Subjects remained at the GCRC after eating their snack and were asked to sleep by 2300 h. Beginning at 0700 h, blood samples were drawn every 10 min until 1300 h for measurement of serum leptin concentrations. After 2 h of baseline blood sampling, subjects began their exercise bout or remained at rest (control day, C). The exercise bout began at 0900 h and continued until 0930 h. During the exercise bout, blood lactate was measured every 10 min and metabolic data were measured minute by minute. Metabolic data were measured minute by minute using open circuit spirometry (SensorMedics 2900Z Metabolic Measurement Cart, Anaheim, CA) during the 30-min exercise bout and during the immediate 30 min post-exercise while the subject sat quietly in the exercise lab. Upon completion of exercise plus 30 min post-exercise, subjects resumed bedrest and metabolic parameters were measured during the last 30 min of each hour (until 1300 h) using a Delta-Trac bedside metabolic unit (SensorMedics) until 1300 h. Subjects were then fed and discharged. All of the above procedures were followed on the non-exercise control day (0700–1300 h) with the exception that at 0900 h the subjects remained in their rooms and metabolic parameters were measured from 0900 to 1000 h and during the last 30 min of each hour using a Delta-Trac bedside metabolic unit (SensorMedics, Anaheim, CA).

Leptin analysis. Serum leptin concentrations were measured in duplicate using a sensitive and specific commercially available radioimmunoassay (Linco Research, St. Charles, MO). Integrated serum leptin concentrations (area under the curve, AUC) were calculated using the trapezoidal rule. Baseline (0700–0900 h), exercise and recovery (0900–1300 h), and total (0700–1300 h) leptin AUC were determined.

Statistical analysis. Analysis of variance with repeated measures was used to determine mean differences for leptin AUC. Regression analyses were also employed to examine the relationship between leptin, fat mass, and $\dot{V}O_2$***peak***. Values are means ± SEM. $P < 0.05$ was chosen a priori.

RESULTS

As expected kcal expended during the 30 min of exercise increased with exercise intensity (150 ± 11, 271 ± 23, 364 ± 28, 439 ± 31, 529 ± 45, kcal for 0.25–1.75 LT, respectively). Similar results were observed for kcal expended during 30 min of exercise + 3.5 h of recovery (310± 14,438±26,551 ± 33, 616 ± 35, 722 ± 51, kcal for 0.25–1.75 LT, respectively).

The effects of exercise intensity on serum leptin concentrations during exercise and recovery are shown in Figure 1. From 0700 to 0900 h, subjects rested quietly, exercise was performed from 0900 to 0930 h, and subjects rested from 0930 to 1300 h. Inspection of Figure 1 reveals no effects of exercise intensity on serum leptin concentrations at any time interval.

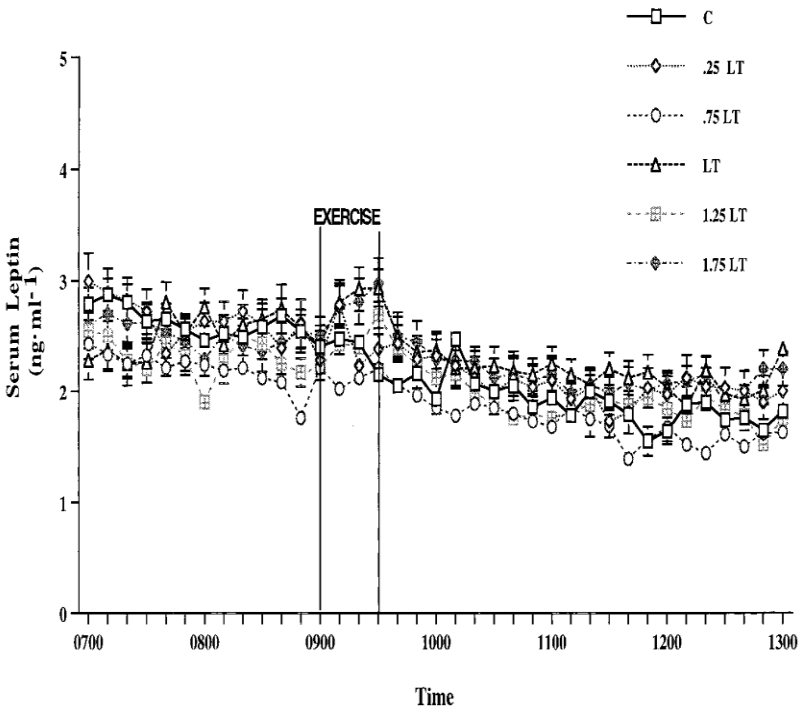

Figure 1—The effects of exercise intensity on serum leptin concentrations (mean ± SEM) during exercise and recovery (*N* = 7). Treadmill velocity was set at 25% and 75% of the difference between the $\dot{V}O_2$ at LT and $\dot{V}O_2$ at rest (.25LT and .75LT, respectively); at LT (LT); and 25% and 75% of the difference between the $\dot{V}O_2$ at LT and $\dot{V}O_2$peak (1.25LT and 1.75LT, respectively). From 0700 to 0900 h subjects rested quietly, exercise was performed from 0900–0930 h, and subjects rested quietly from 0930–1300 h.

Figure 2 shows the integrated serum leptin concentrations for baseline (0700–0900 h) and exercise and recovery (0900–1300 h). Baseline leptin AUC ranged from 243 ± 33 (.75 LT) to 291 ± 56 (.25 LT) ng•mL^{-1} X min, whereas exercise and recovery leptin AUC ranged from 424 ± 56 (.75 LT) to 542 ± 91 (LT) ng•mL^{-1} X min. The latter values normalized to a 2-h epoch

equivalent to baseline conditions were 212 (0.75 LT) to 271 (LT) ng•mL^{-1} X min. No significant differences were observed among conditions within either the baseline or exercise and recovery time frames. In addition, no significant effects of exercise intensity on peak serum leptin concentrations (from 0900 to 1000 h, see Fig. 1) was observed. Peak serum leptin concentration ranged from 2.43 ± 0.30 (.75 LT) to 3.24 ± 0.59 ng•mL^{-1} (P = 0.25).

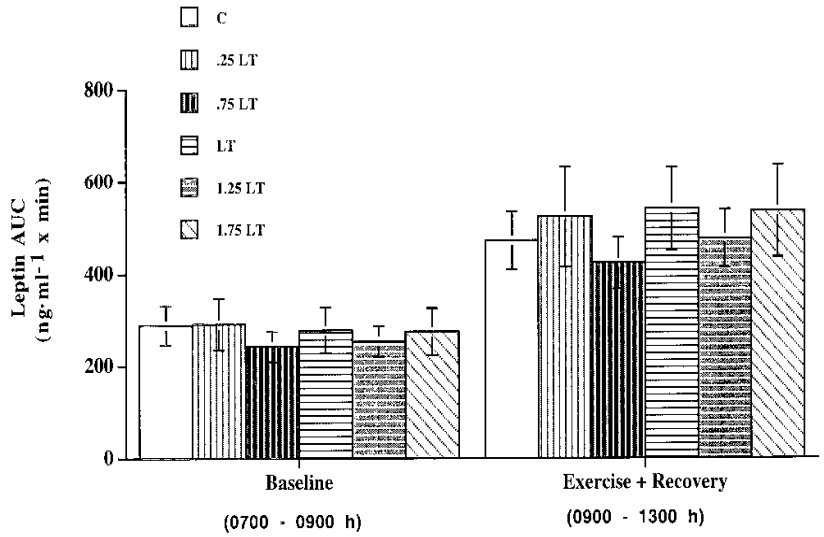

Figure 2—Effects of exercise intensity on integrated serum leptin concentrations (Leptin AUC) (*N* = 7). Velocity was set at 25% and 75% of the difference between the $\dot{V}O_2$ at LT and $\dot{V}O_2$ at rest (.25LT and .75LT, respectively); at LT (LT); and 25% and 75% of the difference between the $\dot{V}O_2$ at LT and $\dot{V}O_2$peak (1.25LT and 1.75LT, respectively). Baseline = 0700–0900 h; exercise and recovery = 0900–1300 h.

Mean serum leptin AUC (mean of the 6 conditions from 0700–1300 h) was strongly related to both percentage body fat (r = 0.94, Fig. 3a) and fat mass (r = 0.93, Fig. 3b). Although a moderate inverse correlation between $\dot{V}O_2$***peak*** and serum leptin AUC was present (r = -0.61), an independent relationship between $\dot{V}O_2$***peak*** and serum leptin was not observed as $\dot{V}O_2$***peak*** was also inversely correlated with percent body fat (r = -0.64) and fat mass (r = -0.67). Body weight did not change over the time course of the study and ranged from 82.1 ± 2.8 kg (admission 1) to 82.8 ± 3.0 kg (admission 4).

DISCUSSION

The administration of recombinant OB protein (leptin) to *ob/ob* mice decreases body fat by decreasing food intake and increasing metabolic rate and physical activity (23). In humans, altering body energy requirements through dietary manipulation also affects serum leptin (5,15,16,19,21,28). Serum leptin falls dramatically with short-term fasting (15,27) and increases with overfeeding (16). Thus, we hypothesized that an increase in energy expenditure, as a result of acute exercise, would also alter serum leptin. Furthermore, as other metabolic hormones are affected by exercise intensity (e.g., catecholamines, GH in a linear dose response manner (7,8,25,30)), and as leptin has been suggested to be a metabolic signal that regulates GH

secretion (3), we postulated that a relationship between leptin and exercise intensity would also exist.

Few studies have examined the relationship between acute exercise and serum leptin in humans. Hickey et al. (10) reported that in male distance runners, serum leptin levels were the same before and after a 20-mile run. Similarly, Racette et al. (26) and Perusse et al. (24) reported that an acute exercise bout did not affect serum leptin levels in non-athletic subjects. In these studies, leptin was only measured before, during, and immediately after exercise. Thus, the possibility that post-exercise alterations in leptin release, related to physical activity itself or negative energy balance could not be examined. Although Landt et al. (17) reported that 2 h of exercise reduced serum leptin by 8.3% in subjects who were fasted overnight, the control condition (fasted overnight + 2 h) also resulted in a reduction in serum leptin (12.3%). However, in the same study, the prolonged exercise of an ultramarathon significantly reduced serum leptin concentration by 32% (17). More recently, Dirlewanger et al. (6) reported that moderate physical activity performed over 3 d does not alter plasma leptin, even when energy balance is slightly negative.

Results of the present study demonstrate that aerobic exercise for 30 min, at intensities ranging from well below to well above the lactate threshold with exercise caloric expenditure ranging from 150 to 529 kcal, does not alter leptin release either during exercise itself or during 3.5 h of recovery thereafter (Figs. 1 and 2). The present data also suggest that a short-term delayed effect of acute exercise on serum leptin levels, regardless of exercise intensity, is not present (Fig. 1). Thus, if a "critical" level of exercise mediated energy expenditure is needed to lower serum leptin levels, acute exercise that results in the expenditure of $\leq$ 529 kcal does not appear to be a sufficient stimulus. These data suggest that typical exercise prescription will not result in acute changes in serum leptin. However, it is possible that the regulation of 24-h plasma leptin levels may be affected by exercise. Recent data of van Aggel-Leijssen et al. (27) indicate that a 28% increase in 24-h energy expenditure induced by exercise (four cycling bouts at 1000 h, 1200 h, 1500 h, and 1700 h) results in a decrease in peak and average 24-h plasma leptin concentration. These effects were not acute but did manifest within 24 h. As we did not collect 24-h leptin samples, it is possible that a downstream (i.e., nighttime) effect of acute exercise on leptin release may have occurred. However, as the increase in energy expenditure that we observed with one 30-min acute exercise bout was considerably lower than the increase in energy expenditure observed with four daily exercise bouts, it is less likely that our subjects would have had leptin alterations later in the day and/or night.

Consistent with data of Landt et al. (17), the slight downward trend in serum leptin over time (Fig. 1) was likely related to the duration of the overnight and morning fast, as no differences were observed between control and exercise conditions. Furthermore, it is unlikely that an affect of exercise on serum leptin would be observed even later, since the administration of a meal post-exercise restores leptin to pre-exercise/pre-fasting levels (17). The present data also support the suggestions that relatively chronic changes in energy expenditure are required to alter the dynamics of leptin production and/or clearance (10), and/or that extremes of exercise-induced negative energy balance (such as the energy expenditure associated with the prolonged exercise of an ultramarathon) are required to lower serum leptin levels (17). Although we were unable to demonstrate an effect of exercise intensity on serum leptin release, the present data are consistent

with the theme that serum leptin is strongly related to % body fat and fat mass (r = 0.94 and r = 0.93, Fig. 3) (5,12).

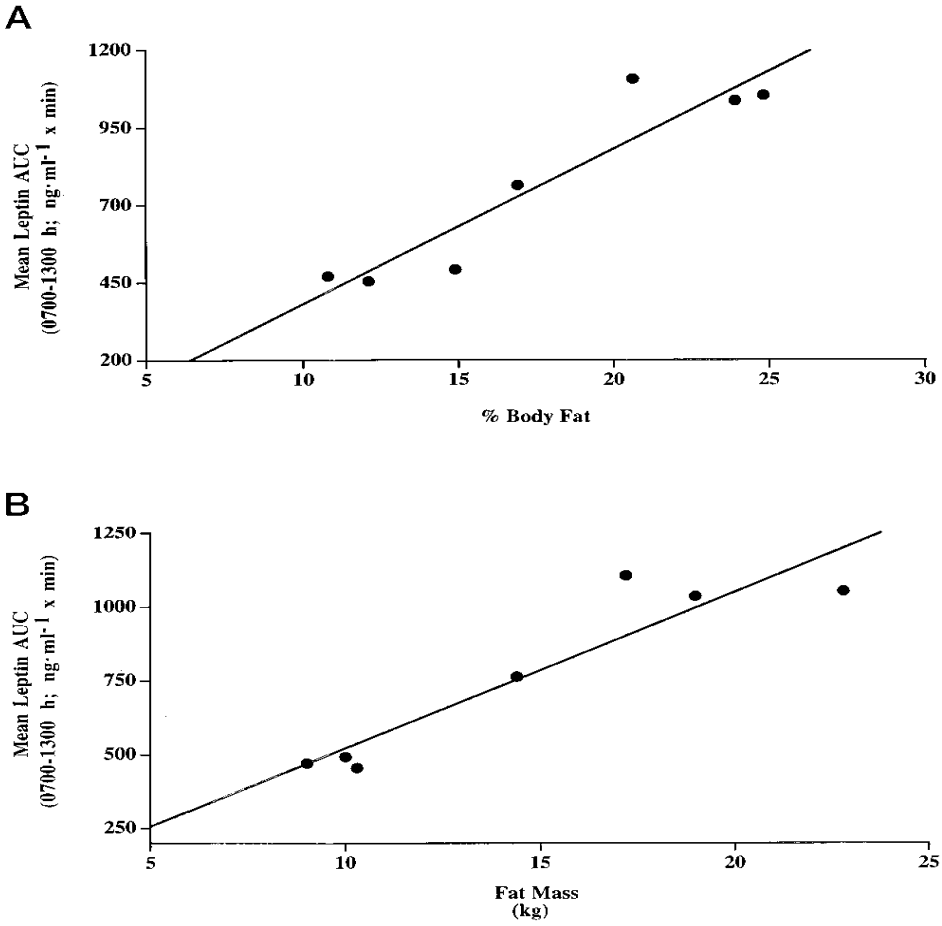

Figure 3—Relationship between mean serum leptin area under the curve (mean of the 6 conditions from 0700–1300 h) and percentage body fat (r = 0.94, a) and fat mass (r = 0.93, b).

The lactate threshold was chosen as the criterion for exercise intensity based on previous data that suggest that release of both catecholamines and GH is stimulated at exercise intensities above the lactate threshold (7,8,25,30). We recently reported (in a slightly larger sample of subjects, the present subjects are a subset of that data set) that GH release associated with acute exercise was related to exercise intensity in a linear dose-response relationship (25). Although it has been suggested that leptin is a metabolic signal that regulates GH secretion in rats (3) and that in normal elderly subjects leptin may provide a signal that mediates the reduction in GH release associated with increased adiposity (9), the present data combined with our recent findings (25) suggest that the GH response to acute exercise is not mediated through leptin as a metabolic signal.

The effects of chronic exercise training on serum leptin concentrations have produced conflicting results, with some investigators suggesting an independent effect of exercise (11,22) (which may or may not be gender dependent) and other authors indicating that the change in serum leptin

observed with exercise training is mediated via a reduction in fat mass (13,24). Pasman et al. (22) recently suggested that the differences observed in leptin response to exercise training may be a result of exercise intensity and duration, with higher intensity and duration of training being important. Because we did not examine chronic exercise training in the present study, our results do not address whether long-term total energy expenditure or exercise intensity are important in affecting change in serum leptin concentrations.

The present data were collected in young men. As leptin production is influenced by gender, at least partially due to differences in reproductive hormones (4), a lack of a relationship between exercise intensity and leptin release in female subjects should not necessarily be inferred from the present data. Similarly, possible effects of aging on the relationship between exercise intensity and serum leptin should be examined separately.

In conclusion, results of the present study indicate that, regardless of exercise intensity, acute exercise for 30-min duration does not affect serum leptin release during exercise and 3.5 h of recovery from exercise in young men. Further studies are needed to evaluate the long-term effects on leptin metabolism of intensity of exercise training, as opposed to total energy expenditure, during exercise training.

REFERENCES

1. AMERICAN COLLEGE OF SPORTS MEDICINE. ACSM's Guidelines for Exercise Testing and Prescription. Baltimore: Williams & Wilkins, 1995, p. 163.
2. BROZEK, J., F. GRANDE, J. T. ANDERSON, and A. KEYS. Densitometric analysis of body composition of men from girth measurements: revision of some quantitative assumptions. Ann. N. Y. Acad. Sci. 1 10(part I):113–140, 1963.
3. CARRO, E., R. SENARIS, R. V. CONSIDINE, F. F. CASANUEVA, and C. DIEGUEZ. Regulation of in vivo growth hormone secretion by leptin. Endocrinology 138:2203–2206, 1997.
4. CONSIDINE, R.V., and J. F. CARO. Leptin: genes, concepts and clinical perspective. Horm. Res. 46:249–256, 1996.
5. CONSIDINE, R. V., M. K. SINHA, M. L. HEIMAN, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292–295, 1996.
6. DIRLEWANGER, M., V. DI VETTA, V. GIUSTI, P. SCHNEITER, E. JEQUIER, and L. TAPPY. Effect of moderate physical activity on plasma leptin concentration in humans. Eur. J. Appl. Physiol. 79:331–335, 1999.
7. FELSING, N. E., J. A. BRASEL, and D. M. COOPER. Effect of low and high intensity exercise on circulating growth hormone in men. J. Clin. Endocrinol. Metab. 75:157–162, 1992.
8. GALBO, H. Hormonal and Metabolic Adaptation to Exercise. New York: Theime-Stratton, 1983, pp. 2–62.
9. GILL, M. S., A. A. TOOGOOD, P. A. O'NEILL, et al. Relationship between growth hormone (GH) status, serum leptin and body composition in healthy and GH deficient elderly subjects. Clin. Endocrinol. 47:161–167, 1997.
10. HICKEY, M. S., R. V. CONSIDINE, R. G. ISREAL, et al. Leptin is related to body fat content in male distance runners. Am. J. Physiol. 272(Endocrinol. Metab. 35):E562–E566, 1997.

11. HICKEY, M. S., J. A. HOUMARD, R. V. CONSIDINE, et al. Gender-dependent effects of exercise training on serum leptin levels in humans. Am. J. Physiol. 271 (Endocrinol. Metab. 34):E938–E940, 1996.
12. KATCH F. I., E. D. MICHAEL, and S. M. HORVATH. Estimation of body volume by underwater weighing: description of a simple method. J. Appl. Physiol. 23:811–813, 1967.
13. KOHRT, W. M., M. LANDT, and S. J. BIRGE, JR. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. J. Clin. Endocrinol. Metab. 81:3980–3985, 1996.
14. KOISTINEN, H. A., J. A. TUOMINEN, P. EBELING, M. L. HEIMAN, T. W. STEPHENS, and V. A. KOIVISTO. The effect of exercise on leptin concentration in healthy men and in type 1 diabetic patients. Med. Sci. Sports Exerc. 30:805–810, 1998.
15. KOLACZYNSKI, J. W., R. V. CONSIDINE, J. OHANNESIAN, et al. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes 45: 1511–1515,1996.
16. KOLACZYNSKI, J. W., J. OHANNESIAN, R. V. CONSIDINE, C. MARCO, and J. F. CARO. Response of leptin to short term and prolonged overfeeding in humans. J. Clin. Endocrinol. Metab. 81:4162– 4165, 1996.
17. LANDT, M., G. M. LAWSON, J. M. HELGESON, et al. Prolonged exercise decreases serum leptin concentrations. Metabolism 46: 1109–1112,1997.
18. LEAL-CERRO, A., P. P GARCIA-LUNA, R. ASTORGA, et al. Serum leptin levels in male marathon athletes before and after the marathon run. J. Clin. Endocrinol. Metab. 88:2376–2379, 1998.
19. MAFFEI, M. J., J. HALAAS, E. RAVUSSIN, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight reduced subjects. Nature 1:1155–1161, 1995.
20. MAZZEO, R., and P. MARSHALL. Influence of plasma catecholamines on the lactate threshold during graded exercise. J. Appl. Physiol. 67:1319–1322, 1989.
21. OSTLUND, R. E., JR., J. W. YANG, S. KLEIN, and R. GINGERICH. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J. Clin. Endocrinol. Metab. 81: 3909–3913, 1996.
22. PASMAN, W. J., M. S. WESTERTERP-PLANTENGA, and W. H. M. SARIS. The effect of exercise training on leptin levels in obese males. Am. J. Physiol. 274(Endocrinol. Metab. 37):E280– E286, 1998.
23. PELLEYMOUNTER, M. A., M. J. CULLEN, M. B. BAKER, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540–543, 1995.
24. PERUSSE, L., G. COLLIER, J. GAGNON, et al. Acute and chronic effects of exercise on leptin levels in humans. J. Appl. Physiol. 83:5–10, 1997.
25. PRITZLAFF, C. J., L. WIDEMAN, J. Y. WELTMAN, et al. Impact of acute exercise intensity on pulsatile growth hormone release in men. J. Appl. Physiol. 87:498–504, 1999.
26. RACETTE, S. B., S. W. COPPACK, M. LANDT, and S. KLEIN. Leptin production during moderate-intensity aerobic exercise. J. Clin. Endocrinol. Metab. 82:2275 –2277, 1997.
27. 27. VAN AGGEL-LEIJSSEN, D. P. C., M. A. VAN BAAK, R. TENENBAUM, L.A. CAMPFIELD, and W. H. M. SARIS. Regulation of average 24 h human plasma leptin

level: the influence of exercise and physiological changes in energy balance. Int. J. Obesity 23:151–158, 1999.
28. WEIGLE, D. S., B. DUELL, W. E. CONNOR, R. A. STEINER, M. R. SOULES, and J. L KUIJPER. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J. Clin. Endocrinol. Metab. 82:561–565, 1997.
29. WELTMAN, A., D. SNEAD, P. STEIN, et al. Reliability and validity of a continuous incremental treadmill protocol for the determination of lactate threshold, fixed blood lactate concentrations, and V˙O2 max. Int. J. Sports Med. 11:26–32, 1990.
30. WELTMAN, A., C. M. WOOD, C. J. WOMACK, et al. Catecholamine and blood lactate responses to incremental rowing and running exercise. J. Appl. Physiol. 76:1144–1149, 1994.
31. WILMORE, J. H. A simplified method for the determination of residual lung volume. J. Appl. Physiol. 27:96–100, 1969.